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LYMPH NODE METASTASES FROM SARCOMA I
IN IMMUNOLOGICALLY ENHANCED AND RESISTANT MICE

Asa Barnes, Jr.


1959

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FROM SARCOMA I
IN "IMMUNOLOGICALLY ENHANCED"
AND RESISTANT MICE

BY

Asa Barnes, Jr.

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INTRODUCTION

Tissue transplants between genetically dissimilar individuals or, more specifically, from one inbred strain to another unrelated inbred strain are known as "homografts". Transplants between genetically uniform animals of the same inbred strain are "isografts". Characteristically, homografts of mature tissue will not grow and isografts will. For exploration of these and related phenomena transplantable tumor grafts, because of the ease with which this tissue can be transplanted, are often the research tool of choice although normal tissue grafts may be preferred in some instances. Highly inbred strains of mice are convenient experimental animals because of the certainty with which genetic factors may be controlled and the relatively low cost per animal. The response of a host to a graft of tumor, or normal tissue, from a genetically dissimilar donor leading to ultimate graft rejection and destruction is known as the "homograft reaction". A host receiving two successive grafts from the same, or genetically similar, foreign hosts rejects the second graft more rapidly than the first. This accelerated reaction to a second graft is termed the "second set response".

Many investigators have found that the "second set response" under certain conditions will not take place. As early as 1907 Flexner and Jobling reported that a second

graft of a transplantable rat sarcoma grew progressively in a large proportion of rats in which an initial graft of the same tumor had previously regressed (1). The first systematic studies on abrogation of the "second set response" were done by Casey and his co-workers with the Brown-Pearce tumor in rabbits and transplantable tumors in mice (2-8). In animals which were pretreated with killed homologous tumor tissues, grafts of tumor were found to grow and metastasize more widely and rapidly. Further, this effect was proved specific, in that it was only produced by killed tissue of the tumor to be transplanted.

Kaliss demonstrated the essential unifying phenomenon in these and similar studies pertaining to the induced survival of tumor homografts. He found that sera produced in mice or rabbits by inoculating killed tissues, or using live transplants, when injected into prospective hosts prior to grafting the tumor would insure survival of the grafts (9-11). By zone electrophoresis and salt fractionation this effect was demonstrated to be due to an antibody in the sera. The active fraction was in the globulin portion, probably the gamma globulin (12). So the paradox of enhanced growth of tumor grafts following prior treatment intended to heighten resistance was the result of exposure of the graft to antiserum produced against it.

This progressive growth of a homograft of a transplantable mouse tumor in a foreign host strain of mice as

a result of the exposure of the graft to antiserum is termed "immunological enhancement" (13,14). The antiserum may be produced by active immunization with lyophilized tumor or liver, kidney or spleen, from the strain of mouse to which the tumor is native (15-19). Passive immunization can be produced by injection of anti-tissue sera (10-12).

Several hypotheses have been advanced as an explanation of the action of antiserum in relation to enhanced growth of the graft. A few are considered by Kaliss and Bryant in their recent paper (20). A theory not discussed by them has been suggested by Snell (21,22) and by Billingham, Brent and Medawar (23). They propose that a "welling off" of the tumor graft by the antiserum may occur which will prevent the tumor cells from antigenically stimulating the host's lymphoid tissue and thus avoid the "cellular response" thought to be responsible for the destruction of the graft (24-26). As Snell says, "The suggestion is that antiserum prevents or delays the antigens of the homograft, or at least effective antigens, from reaching the regional lymph nodes. The nodes are thus unable to generate the cellular immune factor which is the principal agent of graft destruction. Snell has called this a 'welling off' of the graft, and Billingham et al. an 'efferent inhibition' " (22).

It was the purpose of this experiment to test the above hypothesis and, if possible, to shed further light

on the mechanism of "immunological enhancement". If tumor cells could be found in the lymph nodes of immunologically enhanced animals, and particularly if they could be found there within a short time after tumor grafting, then this would be direct proof that the "welling off" or "afferent inhibition" theory is fallacious. If viable tumor cells reach the lymph nodes, no more effective antigenic stimulus could be necessary or even possible. The design of this experiment is similar, with certain technical modifications, to work of Mitchison although he found no viable tumor cells in lymph nodes draining the area of regression of a tumor (25,26).

MATERIALS AND METHODS

The transplantable mouse tumor used was Sarcoma I, which is indigenous to the inbred A strain of mice and grows in solid form when injected subcutaneously and in ascites form when injected intraperitoneally. The strains of mice used were: A/Ks, A/Jax and C57BL/Ks (a C57BL/6 subline). A/Ks and A/Jax are genetically similar, and grafts of Sarcoma I will grow and kill almost 100 per cent of those inoculated within five weeks. The C57BL/Ks strain is normally resistant to grafts of Sarcoma I. In C57BL/Ks hosts Sarcoma I grafts characteristically grow for about 12 days then rapidly regress. All the mice used in this experiment were 4 to 7 months old except for the A/Jax mice used for confirmation of tumors which were 2 to 3 months old.

There were 4 large groups of mice. Group I was composed of C57BL/Ks mice equally divided as to sex. This group received 4 intraperitoneal injections of lyophilized Sarcoma I over a period of 13 days. Each injection was composed of 4 mgm dry weight of the freeze dried tumor in 0.5 ml physiological saline. Ten days after the last injection of lyophilized Sarcoma I each mouse received in the flank a subcutaneous inoculation of 600,000 cells of Sarcoma I. This inoculum was prepared by taking Sarcoma I ascites fluid and counting the cells by the ordinary white cell count procedure and diluting the ascites fluid with physiological saline to the desired concentration of cells.

Group II was composed of C57BL/Ks mice equally divided as to sex. This group received an intraperitoneal injection of 0.5 ml of pooled anti-Sarcoma I sera. These sera were obtained by bleeding C57BL/Ks mice after primary immunization with a solid inoculum of Sarcoma I or bleeding after subsequent booster shots of 1 to 10 homogenates of frozen Sarcoma I. They were prepared by allowing them to remain for 2 hours at room temperature then spinning down the clot and pipetting off the serum which was frozen at -23°C . until used. On the same day as the injection of the anti-Sarcoma I serum these mice were inoculated subcutaneously with 600,000 cells of Sarcoma I prepared as previously described.

Group III was composed of C57BL/Ks mice and Group IV of A/Ks mice. These were equally divided as to sex.

They received no pretreatment, but were inoculated subcutaneously with 600,000 Sarcoma I cells prepared as described above.

Each of these large groups was subdivided into 5 smaller groups composed of 6 males and 6 females each. One of these subgroups from each large group was sacrificed 3,5,7, and 14 days after inoculation with Sarcoma I tumor cells. When the animals were sacrificed, the axillary, brachial and inguinal lymph nodes from the side of implantation of the tumor cells were removed, trimmed free of fat and minced by chopping the nodes finely with scissors in a single drop of physiological saline on a sterile glass slide. This mince was injected through a sterile trocar intraperitoneally after a small transverse incision in the abdominal skin had been made with sterile scissors. All the lymph node inoculations were made into A/Ks mice, a substrain of the A/Jax strain to which Sarcoma I is indigenous. The wound was closed with collodion to minimize infection or possible leakage of the injected material. Occasionally lymph nodes were found overgrown by tumor, and these were discarded and not transferred. Two node donor mice per host were used, and nodes were transferred only from males to a male and from females to a female. The contralateral lymph nodes were treated in the same way as the ipsilateral nodes just described.

The fifth subgroups from each of the larger groups were not sacrificed and the growth of the grafts was

followed by periodic palpation until the mice either died with a progressively growing tumor or remained without an evident sign of growth for a consecutive period of two months at which time they were sacrificed and autopsied. If no evidence of tumor was found, they were classified as negative.

The A/Ks mice which received the lymph nodes were observed daily for the appearance of ascites or solid tumors. When a mouse developed ascites, it was sacrificed and 1 cc of the ascitic fluid was injected subcutaneously into A/Jax mice. Slide preparations were also made of the ascitic cells, and an autopsy was performed on each mouse. The A/Jax mice had to develop solid tumors which grew to their death before the A/Ks mouse was considered to have had tumor cells in the lymph nodes it received. All slides were reviewed and confirmed as showing sheets of tumor cells. The A/Ks mice which did not develop tumors were observed for 3 months and then sacrificed and autopsied to search for any evidence of tumors.

RESULTS

The results of this experiment are given in Table I. Twenty four animals developed tumorous ascites. Each recipient group was composed of 6 males and 6 females except in 3 groups, as noted in Table I, in which animals died of unrelated causes. Of the 24 animals which demon-

strated that the nodes they received contained viable tumor cells, 6 were hosts for node donors in Group I, the "actively enhanced" group; 3 were hosts for node donors in Group II, the "passively enhanced" group; 8 were from Group III, the hosts whose donor's nodes were supposedly resistant; and 7 were from Group IV, the hosts whose donor's nodes were considered maximally susceptible to invasion by tumor cells. Viable tumor cells were present in the contralateral nodes received by 5 animals. As to sex, 12 males and 12 females died with tumorous ascites. In general it developed earlier in females, the first female dying 22 days after receiving nodes, and 7 days later 8 females, as opposed to 3 males, had been killed by the tumor.

TABLE I

Key:

- Column 1: Subgroups
 Column 2: Pretreatment of node doners
 Column 3: Lymph node doner strain
 Column 4: Days from donor tumor inoculation to sacrifice and node transfer
 Column 5: Number of recipient mice dying with tumor / number inoculated with nodes
 Column 6: Number of males dying / number of females dying
 Column 7: Mice dying that received contralateral nodes

1	2	3	4	5	6	7
Group I:						
A	Fr. dr. SaI	C57BL/Ks	3	1/12	1M/OF	0
B	"	"	5	5/12	3M/2F	0
C	"	"	7	0/12	0	0
D	"	"	14	0/11	0	0
E	"	"	not sacrificed (control group)			

Group II:						
F	Anti-SaI serum	C57BL/Ks	3	1/12	1M/OF	0
G	"	"	5	0/12	0	0
H	"	"	7	2/12	2M/OF	0
I	"	"	14	0/12	0	0
J	"	"	not sacrificed (control group)			

Group III:						
K	Nothing	C57BL/Ks	3	3/12	2M/1F	0
L	"	"	5	4/12	3M/1F	1F
M	"	"	7	1/11	1M/OF	0
N	"	"	14	0/12	0	0
O	"	"	not sacrificed (control group)			

Group IV:						
P	Nothing	A/Ks	3	2/12	2M/CF	0
Q	"	"	5	4/12	1M/3F	1M/3F
R	"	"	7	0/12	0	0
S	"	"	14	1/12	1M/OF	0
T	"	"	not sacrificed (control group)			

DISCUSSION

The limits of this tumor-donor-host combination should be defined by the groups composed of A/Ks donors (Group IV, Table I) since this is the strain to which Sarcoma I is native. These maximally susceptible donor animals should have produced the greatest possible number of lymph nodes containing viable tumor cells. As an approximate indication of the sensitivity of the method used in this experiment, Kaliss found that 200 to 500 Sarcoma I cells injected intraperitoneally into A/Ks mice will kill 60 per cent of those injected in 22 to 25 days (Kaliss, unpublished data).

The presence of viable tumor cells in lymph nodes excised from mice in Groups I and II is evidence that tumor grafts in "immunologically enhanced" mice are not "walled off". The finding of metastases as early as 3 days (Subgroups A and F) after tumor inoculation indicates that "afferent inhibition" plays no important role in enhancement. These results make improbable the hypothesis that antiserum prevents or delays effective antigens of the homograft from reaching the regional lymph nodes.

Viable tumor cells being present in the nodes of 8 Group III mice is at variance with the findings of Mitchison who "repeatedly implanted in mice susceptible to the tumor lymph nodes draining the area of regression of a tumor in order to test for the presence of viable tumor

cells" and found "the transferred nodes never gave rise to tumors" (26). Perhaps an explanation of this discrepancy lies in the different methods used to inject lymph nodes. Use of a trocar made possible the injection of the entire inoculum of minced nodes, while enough of the mince might have remained in the 19 gauge needle and the bored out nozzle of the 0.25 ml syringe used by Mitchison so that a critical concentration of tumor cells was not injected. Mitchison does not indicate how many nodes composed an inoculum for a single mouse.

The fact that all of the nodes containing viable tumor cells which were from the contralateral side of the donor were excised on the fifth day after tumor inoculation suggests that there may be a critical time during which hematogenous metastases occur. That lymphatic metastases continue to occur is demonstrated by the animals which received nodes on the seventh and twelfth post inoculation days (Subgroups H, M, and S). These factors probably vary with the host-tumor combination under study.

SUMMARY

Sarcoma I was inoculated into 4 groups of mice:

(1) actively enhanced mice, (2) passively enhanced mice, (3) unenhanced mice of a strain normally resistant to grafts of this tumor and (4) mice of the strain in which this tumor is propagated. The lymph nodes from these mice were excised at 3,5,7, and 14 day intervals after implantation and inoculated into mice to which the tumor is indigenous. Twenty four animals developed tumors, demonstrating the lymph nodes they received contained viable tumor cells. Nine of these mice received nodes from actively or passively enhanced donors proving that sufficient antigenic stimulus is not lacking or delayed in immunologically enhanced animals. This makes it seem unlikely that "walling off" or "afferent inhibition" can be a valid explanation of the mechanism of action of antisera in this phenomenon. Finding viable tumor cells in lymph nodes draining the area of regression of a tumor in eight animals of Group III conflicts with Mitchison's reported results.



An A/Ks mouse with tumorous ascites.



A C57BL/Ks mouse with a subcutaneous tumor.

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